Spatio-temporal control of the assembly of hyaluronan matrices

N. S. Baranova[1], A. J. Day[2], R. P. Richter[1, 3, 4]

[1] Biosurfaces Unit, CIC biomaGUNE, Donostia-San Sebastian, Spain
[2] Wellcome Trust Center for Cell-Matrix Research, University of Manchester, United Kingdom
[3] I2BM, Department of Molecular Chemistry, J. Fourier University, France
[4] Max-Planck-Institute for Intelligent Systems, Stuttgart Germany

Mammalian oocytes are surrounded by several layers of cumulus granulosa cells embedded in a thick cellular coat, the cumulus cell-oocyte complex (COC) matrix. A main component of the COC matrix is the high molecular weight linear polysaccharide hyaluronan (HA). The polyanionic nature of HA promotes formation of a highly hydrated matrix directly after induction of ovulation. The viscoelastic properties of the matrix depend on the interaction between HA and specific HA-binding proteins, hyaladherins. It has been reported that that presence of the proteins TSG-6, IαI and PTX3 is critical for successful ovulation and fertilization [1]. The proteins' expression is temporally regulated and the spatial distribution is likely to be heterogeneous: HA, TSG-6 and PTX3 are synthesized by cumulus cells, while IαI is derived from the serum [2]. The supramolecular organization of the COC matrix as well as the mechanism of matrix stabilization remain unknown.

By conventional biological approaches it is difficult to probe the architecture of HA-matrices. Here, we use a surface-based model system – films of end-grafted HA [3] – to investigate the mechanism of COC matrix assembly. A toolbox of surface sensitive techniques, like quartz crystal microbalance (QCM-D), *in situ* ellipsometry and microinterferometry (RICM), provided a good control on the properties of HA films and their remodelling upon addition of proteins.

We found that TSG-6 alone induced a pronounced collapse of HA films by cross-linking HA chains. The cross-linking entities are TSG-6 oligomers, induced after interaction with HA [4]. Addition of IαI impaired TSG-6 mediated cross-linking. PTX3 did not interact with HA. Contrary to hypotheses in the literature [1, 5], it neither binds to TSG-6 nor IαI-modified HA films. Instead, encounter between all three proteins before interaction with HA is required for incorporation of PTX3. In the presence of all proteins, the HA film appears cross-linked but not collapsed. Based on these results, we propose that the spatio-temporal regulation of HA/protein interactions *in vivo* is responsible for the balance between effective expansion of the COC matrix during ovulation on one hand and matrix stabilization through cross-linking on the other.

1. Scarchilli, L., et al. J Biol Chem, 2007. 282(41): p. 30161-70. **2.** Russell, D.L. and A. Salustri, 2006. 24(4): p. 217-27. **3.** Richter, R.P., et al., J. Am. Chem. Soc., 2007. 127(17): p. 5306-5307. **4.** Baranova, N.S., et al., J Biol Chem., 2011. 286(29): p. 25675-86. **5.** Fulop, C., et al.,. Development, 2003. 130(10): p. 2253-61.